Graduate School Research Meeting Presentation

−大学院歯学独立研究科−
第 75 回大学院研究科発表会プログラム
第 88 回中間発表会プログラム

大学院学生等が、これまでの研究成果を発表します。
どなたでも聴講できますので、多数の参加をお待ちしております（聴講申込不要）

場　所：実習館 2 階　総合歯科医学研究所セミナー室
日　時：2017 年 11 月 22 日（水）17 時 25 分　開会

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No.	発表区分・予定時間	演題名・発表者	審査委員
	17：25	開会挨拶　高橋研究科長	
1	[研究科発表] 17：30〜18：00 司会：増田教授	「外耳道のひずみで咀嚼回数をカウントする方法」 菅生 秀昭　4 年　顎口腔機能制御学講座　咀嚼機能解析学	主査：金　銅 教授 副査：十　川 教授 岡　田 准教授
2	[中間発表] 18：00〜18：30 司会：宇田川教授	「骨髄間質細胞層の頂点に位置するレプチン受容体陽性細胞は骨形成因子 Runx2 を発現する」 楊 孟雨　3 年　硬組織疾患制御再建学講座 硬組織機能解析学	主査：川　上 教授 副査：八　上 准教授 荒　講師

問合わせ先：本館 2 階　学事室（大学院）内線 2331

発表内容の要旨(課程博士)
Abstract of Presented Research (For the Doctoral Course)

学籍番号 Student ID No.	ID＃G 1508	入学年 Entrance Year	2015 年 Year
（ふりがな）	やん もんいう		
氏名 Name in Full	楊 孟雨		
専攻分野 Major Field	硬組織疾患制御再建学講座 硬組織機能解析学		
主指導教員 Chief Academic Advisor	宇田川 信之		
発表会区分 Type of Meeting	(中間発表会)・ 大学院研究科発表会 ・ 松本歯科大学学会 Midterm Meeting / Graduate school research meeting presentation /The Matsumoto Dental University Society		

演題名 / Title of Presentation

骨髄間質細胞層の頂点に位置するレプチン受容体陽性細胞は骨形成因子 Runx2を発現する

Osteogenic Factor Runx2 Marks a Subset of Leptin Receptor-Positive Cells that Sit Atop the Bone Marrow Stromal Cell Hierarchy

発表要旨 / Abstract

Purpose:
Bone marrow mesenchymal stem and progenitor cells (BM-MSPCs) maintain homeostasis of bone tissue by providing osteoblasts (*Annu Rev Immunol* 31:285-316, 2013). Although several markers have been identified for labeling of MSPCs, these labeled cells still contain non-BM-MSPC populations. Studies have suggested that MSPCs are observed as leptin receptor (LepR)-positive cells, whereas osteoblasts can be classified as positive for Runx2, a master regulator for osteoblastogenesis (*Dev Cell* 29:340-9, 2014). On the other hand, it is known that bone volume is increased by intermittent parathyroid hormone (1-34) [iPTH(1-34)] treatment due to accelerated the bone formation (*J Bone Miner Res* 17:808,2002). However it is unclear whether the PTH anabolic effect is exerted by mediating the BM-MSPCs. In this study, we will try to understand the questions using by genetic mice.

Method:
1. Analysis of $LepR^+$ cells in the bone marrow cavity express Runx2.
We used Runx2-GFP reporter mice, in which GFP is driven by a bacterial artificial chromosome (BAC) of Runx2 locus (*J Bone Miner Res* 29:1960-1969, 2014). FITC-conjugated anti-GFP antibody was used to amplify the intensity of the GFP signal when imaging bone tissue section. After cryosections made, we analyzed the Runx2 expression in $LepR^+$ cells.
2. Analysis of $LepR^+$ cells contain Runx2-GFP^{low} and Runx2-GFP- sub-population.
We used Runx2-GFP reporter mice. The bone marrow was gently flushed in L-15 FACS buffer. BM was digested with 0.1% collagenase IV, 0.2% Dispase and 20 U/ml DNase in HBSS. We did the cell sorting and flow cytometry.
3. Analysis of Stromal stem cell activity in BM in enriched in $LepR^+$Runx2-GFP^{low} population.
We used LepR-cre mice, in which Leptin Receptor (LepR) positive cells have characteristics of BM-MSCs in vivo by fate mapping approach. (*Dev Cell* 29:340-9, 2014). We did CFU-F assay, Spheroid formation assay and RNA isolation and quantitative real-time PCR.
4. Analysis of $LepR^+$Runx-GFP^{low} cells differentiate into osteoblasts through multilayered cell formation in response to PTH-induced anabolic effects.
We generated LepR-Cre/Tomato/Runx2-GFP mice, which $LepR^+$ cells-derived osteoblasts were observed as yellow cells. We used tamoxifen-administered iOsx/Tomato/Runx2-GFP mice, which mature osteoblasts express both iOsx/Tomato and Runx2-GFP. We also generated iOsx/Tomato/Col1(2.3)-GFP mice, which Col1(2.3)-GFP was a marker of mature osteoblasts. We made cryosections by these three kinds mice.

Results:
1. Runx2 is heterogeneously expressed in the $LepR^+$ BM stromal cell population.
2. Stem cell activity is enriched in the Runx2-GFPlow sub-population of $LepR^+$ cells.
3. $LepR^+$Runx2-GFP^{low} cells differentiate into osteoblasts through multilayered cell formation in response to PTH anabolic effects.
4. $LepR^+$Runx2-GFP^{low} cell-derived multilayered cells differentiate into mature osteoblasts with increasing expression of Osterix and type I collagen α.

Conclusion:
Runx2 is weakly expressed in the $LepR^+$ population without osteoblastic commitment, and the $LepR^+$Runx2-GFP^{low} stromal cells sit atop the BM stromal hierarchy.